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(54) Title: VACCINE FOR *MORAXELLA CATARRHALIS*

(57) Abstract

Compositions comprising outer membrane protein "E", and peptides and oligopeptides thereof, of *Moraxella catarrhalis* are described. Additionally, nucleotide sequences encoding the protein, peptide, or oligopeptide are disclosed, as well as recombinant vectors containing these sequences. Protein, peptide, or oligopeptide can be produced from host cell systems containing these recombinant vectors. Peptides and oligopeptides can also be chemically synthesized. Disclosed are the uses of the protein, peptides and oligopeptides as antigens for vaccine formulations, and as antigens in diagnostic immunoassays. The nucleotide sequences are useful for constructing vectors for use as vaccines, for insertions into attenuated bacteria in constructing a recombinant bacterial vaccine and for inserting into a viral vector in constructing a recombinant viral vaccine. Also described is the use of nucleotide sequences related to the gene encoding E as primers and/or probes in molecular diagnostic assays for the detection of *M. catarrhalis*.

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VACCINE FOR MORAXELLA CATARRHALIS

This invention was made with government support under grant A128304 awarded by the National Institutes of Health, and support by the Department of Veteran Affairs. The government has certain rights in the 5 invention.

Field of Invention

The present invention relates to compositions comprising a protein, and peptides and oligopeptides thereof, associated with the outer membrane of *Moraxella catarrhalis* (previously referred to as *Branhamella catarrhalis*). More particularly, the invention is directed to compositions of a protein, peptides, and oligopeptides thereof, related to an outer membrane 10 protein, "E", which is a heat-modifiable protein of *M. catarrhalis* having an apparent molecular mass of about 35,000 daltons at 25°C and about 50,000 daltons when heated to 100°C. Also disclosed is methods for preparing 15 E, E peptides and E oligopeptides using recombinant DNA and/or biochemical techniques. Related thereto, disclosed is the DNA sequence encoding E, and vectors useful in directing the expression of E, E peptides, and E oligopeptides, and host cells transformed with such 20 vectors.

25 The proteins, peptides, and oligopeptides can be used as immunogens in vaccine formulations for active immunization; and can be used to generate protein-specific and peptide-specific antisera useful for passive immunization, and as reagents for diagnostic 30 assays. The nucleotide sequences disclosed provide for the synthesis of corresponding oligonucleotides which can be used as reagents in diagnostic assays directed to the detection of *M. catarrhalis* genetic material.

Background of the Invention

5 *Moraxella catarrhalis* (also known as *Branhamella catarrhalis*) is an important human respiratory tract pathogen. *M. catarrhalis* is the third most common cause of otitis media in infants and children, after *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*, as documented in studies in which tympanocentesis has been used to establish the etiologic 10 agent (Murphy, 1989, *Pediatr. Infect. Dis. J.* 8:S75-S77). *M. catarrhalis* is a common cause of sinusitis and conjunctivitis in both children and adults (See for example, Bluestone, 1986, *Drugs* 31:S132-S141; Brorson et al., 1976, *Scand. J. Infect. Dis.* 8:151-155; and 15 Romberger et al., 1987, *South. Med. J.* 80:926-928); and is an important cause of lower respiratory tract infections in adults with chronic bronchitis and chronic obstructive pulmonary disease (Murphy et al., 1992, *Am. Rev. Respir. Dis.* 146:1067-1083; Catlin, 1990, *Clin. Microbiol. Rev.* 3:293-320). Additionally, *M. catarrhalis* can cause pneumonia, endocarditis, septicemia, and meningitis in immunocompromised hosts (Cocchi et al., 20 1968, *Acta Paediatr. Scand.* 57:451-3; Douer et al., 1977, *Ann. Intern. Med.* 86:116-119; McNeely et al., 1976, *Am. Rev. Respir. Dis.* 114:399-402).

Since recurrent otitis media is associated with substantial morbidity, there is interest in identifying strategies for preventing these infections. One such approach is the development of vaccines. An effective 30 vaccine for preventing bacterial otitis media would need to include antigens which would generate protection against infection by *S. pneumoniae*, nontypeable *H. influenzae* and *M. catarrhalis*. Indeed, vaccine development for the pneumococcus and nontypeable *H. influenzae* are progressing such that potentially protective antigens have been identified and are 35

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currently undergoing testing (See for example, Murphy et al., U.S. Patent No. 5,173,294; and Vella et al., 1992, *Infect. Immun.* 60:4977-4983). As these vaccines are developed and used more widely, the relative importance 5 of *M. catarrhalis* as a cause of otitis media will increase in the next decade. Besides infants and children benefitting from a vaccine to prevent otitis media caused by *M. catarrhalis*, adults with chronic obstructive pulmonary disease, and immunocompromised 10 children and adults would benefit from a vaccine to prevent infections caused by *M. catarrhalis*.

Bacterial components which have been investigated as potential vaccine antigens include polysaccharides, lipopolysaccharides or modifications thereof, and outer 15 membrane proteins. In general, as exemplified by the type b capsular polysaccharide of *H. influenzae*, polysaccharide antigens have been shown to be a poor immunogen in children under the age of 18 months. Active immunization with lipopolysaccharide (LPS) is 20 unacceptable due to its inherent toxicity. The pathophysiologic effects of LPS may include fever, leucopenia, leucocytosis, the Shwartzman reaction, disseminated intravascular coagulation, and in large doses, shock and death. In general, proteins are 25 immunogenic in infants around three months of age. Thus, outer membrane proteins are being investigated as possible vaccine antigens.

While recent studies have begun to focus on outer membrane proteins of *M. catarrhalis*, little is known 30 about the antigenic and molecular structure of these proteins. Studies of purified outer membranes by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have revealed a rather homogeneous pattern among strains of the bacterium (Bartos and Murphy, 1988, 35 *J. Infect. Dis.* 158:761-765). Eight major outer membrane proteins, designated by the letters A-H, have

been identified (Murphy et al., 1989, *Microbial Pathogen.* 6:159-174; Bartos et al., 1988, *J. Infect. Dis.* 158: 761-765). Experiments in which 20 strains of *M. catarrhalis* were absorbed with antisera developed 5 against *M. catarrhalis* strain 25240 indicate that outer membrane protein E contains antigenically conserved determinants that are expressed on the bacterial surface (Murphy et al., 1989, *Infect. Immun.* 57:2938-2941).

Hence, with the increasing recognition of *M. catarrhalis* as an important bacterial pathogen, there is 10 a need for a vaccine that is immunogenic in children and adults. Such a vaccine would have to be directed to a bacterial component which has a surface-exposed epitope on intact bacteria, wherein the epitope is conserved 15 amongst strains of *M. catarrhalis*.

Summary of the Invention

The present invention is directed to a protein, peptides, and oligopeptides related to an outer membrane 20 protein having an apparent molecular mass of about 35,000 daltons to about 50,000 daltons of *M. catarrhalis*, wherein the protein appears to be a heat-modifiable protein resulting in differences in migration in SDS gels, depending on the sample processing 25 temperature. The E protein, and peptides thereof (herein also termed "E peptides" or "E oligopeptides"), of the present invention may be used as immunogens in prophylactic and/or therapeutic vaccine formulations; or as an antigen in diagnostic immunoassays directed to 30 detection of *M. catarrhalis* infection by measuring an increase in serum titer of *M. catarrhalis*-specific antibody. Also, E protein, E peptides and E oligopeptides of the present invention may be used to generate E-specific antibody which may be useful for 35 passive immunization and as reagents for diagnostic assays directed to detecting the presence of *M.*

catarrhalis in clinical specimens. E peptides or E oligopeptides can be obtained by chemical synthesis; purification from *M. catarrhalis*; or produced from recombinant vector expression systems using the nucleic acid sequences disclosed herein.

One embodiment of the present invention is directed to the construction of novel DNA sequences and vectors including plasmid DNA, and viral DNA such as human viruses, animal viruses, insect viruses, or bacteriophages which can be used to direct the expression of E protein, E peptides, or E oligopeptides in appropriate host cells from which the expressed protein or peptides may be purified.

Another embodiment of the present invention provides methods for molecular cloning of the gene encoding E, and provides compositions comprising oligonucleotides within the gene sequence encoding E. The nucleic acid sequences of the present invention can be used in molecular diagnostic assays for *M.*

catarrhalis genetic material through nucleic acid hybridization, and including the synthesis of E sequence-specific oligonucleotides for use as primers and/or probes in amplifying, and detecting amplified, nucleic acids.

Additionally, E protein, E peptides, and E oligopeptides can be used as immunogens in prophylactic and/or therapeutic vaccine formulations against pathogenic strains of *M. catarrhalis*, whether the immunogen is chemically synthesized, purified from *M. catarrhalis*, or purified from a recombinant expression vector system. Alternatively, the gene encoding E, or one or more gene fragments encoding E peptides or E oligopeptides, may be incorporated into a bacterial or viral vaccine comprising recombinant bacteria or virus which is engineered to produce one or more immunogenic

epitopes of E by itself, or in combination with immunogenic epitopes of other pathogenic microorganisms.

In addition, the gene encoding E or one or more gene fragments encoding E peptides or E oligopeptides, operatively linked to one or more regulatory elements, can be introduced directly into humans to express protein E, E peptide, or E oligopeptides to elicit a protective immune response.

10 **Brief Description of the Figures**

FIG. 1 is a Kay-Doolittle hydrophobicity profile of outer membrane protein E of *M. catarrhalis* as determined using the amino acid sequence deduced from the nucleotide sequence of the gene encoding E. Positive values represent hydrophobic regions and negative values represent hydrophilic regions.

FIG. 2 represents polyacrylamide gels stained with ethidium bromide and containing amplified product from the genomes of different strains of *M. catarrhalis* after digestion with various restriction enzymes. Lane 1 represents DNA size standards, and lanes 2-20 are amplified products from strains listed in Table 1, respectively.

25 FIG. 2A is a gel showing the amplified products restricted with *Sau96 I*.

FIG. 2B is a gel showing the amplified products restricted with *Bsl I*.

30 **Detailed Description of the Invention**

The present invention is directed to compositions of a bacterial outer membrane protein, and peptides thereof, of *M. catarrhalis* wherein the protein has been designated "E". The pattern of migration on SDS-PAGE of the E protein is characteristic of a heat-modifiable protein. That is, the migration pattern depends on the

prior sample processing temperature. Thus, if the sample containing E protein is heated at 25°C prior to SDS-PAGE, the apparent molecular mass is about 35,000 daltons; and if the sample is heated to 100°C, the 5 apparent molecular mass is about 50,000 daltons. As indicated by the nucleotide sequence of the present invention (SEQ ID NO:11), the gene encoding E reveals that the predicted amino acid sequence of the mature E protein has a calculated molecular mass of about 47,030 10 daltons. The E protein, E peptides, and E oligopeptides of the present invention can be produced using recombinant DNA methods as illustrated herein, or can be synthesized chemically from the amino acid sequence disclosed in the present invention. Additionally, 15 peptides can be produced from enzymatic or chemical cleavage of the mature protein. E protein, E peptides, and E oligopeptides with an immunogenic epitope(s), can be used as immunogens in various vaccine formulations in the prevention of otitis media, sinusitis, 20 conjunctivitis, and lower respiratory tract infections caused by *M. catarrhalis*. Additionally, according to the present invention, the E protein, E peptides, and E oligopeptides produced may be used to generate *M. catarrhalis*-specific antisera useful for passive 25 immunization against infections caused by *M. catarrhalis*.

The present invention further provides the nucleotide sequence of the gene encoding E, as well as the amino acid sequence deduced from the isolated gene. 30 According to one embodiment of the present invention, using recombinant DNA techniques the gene encoding E, or gene fragments encoding one or more E peptides having an immunogenic epitope(s), is incorporated into an expression vector, and the recombinant vector is 35 introduced into an appropriate host cell thereby directing the expression of these sequences in that

particular host cell. The expression system, comprising the recombinant vector introduced into the host cell, can be used (a) to produce E protein, E peptides, or E oligopeptides which can be purified for use as an 5 immunogen in vaccine formulations; (b) to produce E protein, E peptides or E oligopeptides to be used as an antigen for diagnostic immunoassays or for generating *M. catarrhalis*-specific antisera of therapeutic and/or diagnostic value; c) or if the recombinant expression 10 vector is a live virus such as vaccinia virus, the vector itself may be used as a live or inactivated vaccine preparation to be introduced into the host's cells for expression of E or immunogenic E peptides or E oligopeptides; d) or if the recombinant expression 15 vector is introduced into live attenuated bacterial cells which are used to express E protein, E peptides or E oligopeptides to vaccinate individuals; e) or introduced directly into an individual to immunize against the encoded and expressed E protein, E peptide, 20 or E oligopeptide.

For purposes of the description, the methods and compounds of the present invention will be illustrated in the following embodiments:

Embodiment A- Molecular cloning and sequencing of the 25 gene encoding E, and vectors expressing E-specific epitopes;

Embodiment B- Conservation of the gene encoding E amongst *M. catarrhalis* strains;

Embodiment C- Methods for using E-specific nucleotide 30 sequences in molecular diagnostic assays for the detection of *M. catarrhalis*;

Embodiment D- Methods for making and using E, E peptides, and E oligopeptides, in diagnostic immunoassays;

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Embodiment E- Methods and compounds for vaccine formulations related to E, E peptides, and E oligopeptides.

5

Embodiment A

Molecular cloning and sequencing of the gene encoding E, and vectors expressing E-specific epitopes.

The strategy used was to isolate genomic DNA from
10 *M. catarrhalis*, cleave the isolated DNA into fragments, construct a genomic library comprising insertion of the fragments into an expression vector, introduce the recombinant vectors into the appropriate host cell, and screen for host cell clones containing the gene encoding
15 E by filter-hybridization with a family of degenerate, labeled oligonucleotides corresponding to the amino terminal sequence of the E protein. The synthesized oligonucleotides were prescreened by Southern blot to *M. catarrhalis* DNA, and *E. coli* as a control, to determine
20 which degenerate oligonucleotides hybridized strongly to *M. catarrhalis* DNA.

Moraxella catarrhalis strain 25240, obtained from the American Type Culture Collection (ATCC) was used as the source of bacterial genomic DNA. *M. catarrhalis* was
25 grown on chocolate agar plates at 37°C in 5% CO₂ or in brain heart infusion broth. *Escherichia coli* (*E. coli*) LE392 was used as the host strain for the bacteriophage lambda (EMBL-3) genomic library. Depending on the circumstances, *E. coli* was grown in tryptone broth
30 supplemented with 0.2% maltose and 10mM MgSO₄; or for screening, on NZCYM agar plates containing 50 µg/ml of ampicillin.

An EMBL3 genomic library was constructed with genomic DNA of *M. catarrhalis* 25240 using previously
35 described methods (Ausubel et al., 1989, Current Protocols in Molecular Biology, published by John Wiley

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and Sons). Genomic DNA of *M. catarrhalis* strain 25240 was purified using detergent extraction, and proteinase treatment. The purified genomic DNA was then partially digested with restriction enzyme *Sau* 3A to generate
5 fragments varying in size. The DNA fragments were separated by sucrose gradient centrifugation on a 10% to 40% sucrose gradient. Fractions containing fragments of approximately 9 to 23 kilobases (kb) in size were collected, dephosphorylated using calf intestinal
10 phosphatase, and subsequently ligated to phage arms and then packaged into phage. A portion of the resultant EMBL-3 library was plated on NZCYM plates with *E. coli* LE392 as the host strain.

Plaques were transferred onto nitrocellulose filter
15 discs and screened by hybridization with a family of degenerate radiolabelled oligonucleotides (representative examples disclosed in SEQ ID NO:1-SEQ ID NO:8) corresponding to the amino terminal sequence of outer membrane protein E. A total of about 8100 plaques
20 were screened and six positive clones were identified. The initial positive plaques were picked, eluted into buffer, and then purified by plating at low density and rescreened with the same oligonucleotides until all the plaques from a rescreening were positive. Liquid lysates
25 of the positive clones were used to isolate the lambda DNA containing the insert. The isolated lambda DNA was then digested with *Sal* I and the digests were electrophoresed on agarose gels to confirm the presence of inserts. Insert sizes of the positive clones were
30 between 12 kilobases (kb) and 17 kb. The clone containing the 12 kb insert was used to localize the gene encoding E contained within the insert. The DNA from the clone was cut with *Sal* I and the 12 kb insert was electroeluted from gel slices and restricted with
35 one or more of several different enzymes (*Nde* I, *Nco* I, *Hind* III, *Sac* I, *Eco* RI, and *Nde* I and *Nco* I). The

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digests were electrophoresed on agarose gels, and the fragments were analyzed by Southern blot with the oligonucleotide probes.

A 4.4 kb *Nde* I-*Sal* I fragment and a 1.9 *Nco* I-*Sal* I fragment were selected and manipulated for subcloning into either of the plasmids pET22b⁺ or pGEM5zf⁻ to facilitate subsequent sequencing. After repeated unsuccessful attempts to transform *E. coli* with the recombinant plasmids, and despite success with control DNA and transformation controls, it was concluded that the fragments containing the gene encoding E, or containing portions thereof, were toxic to the *E. coli*. Thus, an alternative approach was taken to determine the nucleotide sequence. The sequences of the ends of the 1.9 kb fragment were determined by the method of Maxam-Gilbert. The 1.9 kb fragment was digested with *Hind* III and two fragments were purified, a 1.1 kb fragment and a 0.8 kb fragment. These fragments were labelled and then sequenced using the Maxam-Gilbert method (1977, Proc. Natl. Acad. Sci. USA 74:560-564). From this sequence analysis, two additional oligonucleotides were synthesized (SEQ ID NO:9 and SEQ ID NO:10).

Two primers (SEQ ID NO:7 and SEQ ID NO:10) were selected to amplify a fragment of the insert DNA, of the clone having the 12 kb insert, using polymerase chain reaction. The reactions were carried out in a 50 μ l volume with 0.25 μ g of primers and 2.5 mM dNTP. Pre-denaturing was done at 95°C for 3 minutes. Denaturing was done at 96°C for 15 seconds, annealing at 62°C for 1 minute and polymerization for 74°C for 1 minute, for 15 cycles in the presence of 3mM MgSO₄. The result of the polymerase chain reaction using these two primers was an amplified product of 0.8 kb. The 0.8 kb amplified product was purified by agarose gel electrophoresis and electroelution, and then subcloned into the *Eco* RI site of M13mp18. Single-stranded M13 DNA was prepared from

the recombinant to determine the nucleotide sequence of the 0.8 kb product by dideoxy-chain termination method. The remaining portion of the gene encoding E was sequenced directly from the 12 kb insert of the lambda 5 clone using additional oligonucleotides synthesized to correspond to the gene region encoding E.

From the complete nucleotide sequence (SEQ ID NO:11), the gene encoding E is defined as an open reading frame of 1377 base pairs (encoding 460 amino acids) starting with the codon at position 154 and ending with TAA at position 1531. A potential ribosome binding site GGAGA was located five bases upstream of the ATG translation initiation codon. Thirty bases downstream of the TAA stop codon was the sequence 15 ATAAAAAAATAGCTTGAATTCAAGCTATTTTTAT, a palindrome that could form a stem loop structure which potentially serves as a transcriptional terminator. The overall guanine and cytosine (G+C) content of the gene encoding E is 43.4% which is similar to the reported G+C content 20 of 41% for the *M. catarrhalis* genome (Catlin, 1990, *Clin. Microbiol. Rev.* 3:293-320).

The amino acid sequence, deduced from the open reading frame, defined E as a protein of a calculated molecular mass of 49,334 daltons. The amino acid 25 sequence deduced for E suggested the presence of a signal peptide with a probable cleavage site between amino acids 25 (Ala) and 26 (Ala). The first 24 amino acids from the putative cleavage site, of the amino acid sequence deduced from the open reading frame, corresponds precisely to the N-terminal protein sequence determined from the purified outer membrane protein E. These observations further confirm that the gene encodes E, and that E is synthesized as a precursor possessing a signal peptide composed of 25 amino acid residues. A 30 hydrophobicity profile of the deduced amino acid sequence (Fig. 1) showed a strong hydrophobic portion 35

corresponding to the signal peptide. The predicted antigenic determinants correspond to the hydrophilic regions indicated in Fig. 1. These antigenic determinants include amino acids 369 to 374; 29 to 34; 5 and 294 to 299. The predicted molecular mass of the mature protein is 47,030 daltons, which correlates well with the migration of outer membrane protein E in SDS-PAGE of samples containing *M. catarrhalis*. Analysis of the amino acid composition of E indicated that alanine, 10 glycine, leucine and valine are the most abundant (range 13%-18%) and no cysteine residues are present.

To determine the transcriptional initiation site of the gene encoding E, primer extension analysis was performed using two different E-specific primers (SEQ ID NO:12 and SEQ ID NO:13) hybridizing to the 5' region of the corresponding mRNA. Total RNA was extracted by the guanidine thiocyanate method from *M. catarrhalis* strain 25240. The E-specific primers were 5' end labeled with [³²P]ATP. For primer extension, 50 µg of the total RNA 15 was annealed with 100 fmols of the labeled primers and incubated at 55° C for 45 minutes. This was followed by extension with reverse transcriptase in the presence of deoxyribonucleoside triphosphates for one hour at 42°C. The primer extension product was analyzed on an 8% urea 20 acrylamide sequencing gel. Dideoxy nucleotide sequencing reactions generating a sequencing ladder and primed with the same primers were also electrophoresed in adjacent lanes to assess the exact base for the initiation of the transcript corresponding to E. The results indicate 25 that the transcript starts with a guanine residue at position 75 which is 78 bases upstream of the ATG codon. The potential -10 TAAAGAT or the Pribnow box (nucleotide position 63-68) was located six bases upstream of the +1 start site of transcription. The -35 (position 40-45) 30 TTGTT was located seventeen bases upstream of the -10 sequence. Two regions of hyphenated dyad symmetry, 5'-

TTAATTCATTTAA-3' and 5'TACAAATGTGTAAGACTTTGTA-3', were identified downstream of the -35 region which may play a role in regulation of expression of the gene encoding E.

Based on the nucleotide sequence of the gene encoding E, three sets of oligonucleotide primers were synthesized and used to amplify portions of the gene, by polymerase chain reaction, for subcloning and analysis of expression. Two primers (SEQ ID NO:14 and SEQ ID NO:15) were used to amplify 1.573 kb of the gene, the amplified fragment containing the complete gene and the promoter region. Another set of primers (SEQ ID NO:16 and SEQ ID NO:17) were used to amplify 1.391 kb of the gene which contained sequence encoding the leader peptide along with the rest of the gene. A third set of primers (SEQ ID NO:17 and SEQ ID NO:18) were used to amplify 1.313 kb of the gene encoding from the first amino acid of the mature protein to the end of the carboxy terminus. The three amplified products, 1.573 kb, 1.391 kb, and 1.313 kb, were separately subcloned into a vector, phagemid pCR-Script SK⁺, and transformed into *E. coli* using standard protocols. Attempts at transformation with the recombinant plasmid containing the 1.573 kb fragment were unsuccessful suggesting, again, that expression of *M. catarrhalis* protein E in *E. coli* is toxic to the transformed bacteria. Transformants were identified that contained recombinant plasmids with the 1.391 kb insert (the entire open reading frame without the promoter) and the 1.313 kb insert (sequence encoding the mature protein). Confirmation of the inserts, by sequencing the ends of the inserts, indicated that all identified clones contained the gene sequences in the wrong orientation for expression of the protein by the plasmid promoter; further evidence that the expression of protein E is toxic to *E. coli*.

Thus, this embodiment illustrates that nucleotide sequences encoding E or portions thereof, can be

inserted into various vectors including phage vectors and plasmids. Successful expression of the protein and peptides of protein E requires that either the insert comprising the gene or gene fragment which encodes 5 epitopes of protein E, or the vector itself, contain the necessary elements for transcription and translation which is compatible with, and recognized by the particular host system used for expression. DNA encoding E protein, E peptides, or E oligopeptides can be 10 synthesized or isolated and sequenced using the methods and primer sequences as illustrated according to Embodiments A, B, and E herein. A variety of host systems may be utilized to express E protein, E peptides or E oligopeptides, which include, but are not limited 15 to bacteria transformed with a bacteriophage vector, plasmid vector, or cosmid DNA; yeast containing yeast vectors; fungi containing fungal vectors; insect cell lines infected with virus (e.g. baculovirus); and mammalian cell lines transfected with plasmid or viral 20 expression vectors, or infected with recombinant virus (e.g. vaccinia virus, adenovirus, adeno-associated virus, retrovirus, etc.).

Using methods known in the art of molecular biology, including methods described above, various 25 promoters and enhancers can be incorporated into the vector or the DNA sequence encoding E amino acid sequences, i.e. recombinant outer membrane protein E, E peptide or E oligopeptide, to increase the expression of E amino acid sequence, provided that the increased 30 expression of the E amino acid sequences is compatible with (for example, non-toxic to) the particular host cell system used. Thus and importantly, the DNA sequence can consist of the gene encoding E protein, or any segment of the gene which encodes a functional epitope 35 of the E protein. Further, the DNA can be fused to DNA encoding other antigens, such as other bacterial outer

membrane proteins, or other bacterial, fungal, parasitic, or viral antigens to create a genetically fused (sharing a common peptide backbone) multivalent antigen for use as an improved vaccine composition.

5 The selection of the promoter will depend on the expression system used. Promoters vary in strength, i.e. ability to facilitate transcription. Generally, for the purpose of expressing a cloned gene, it is desirable to use a strong promoter in order to obtain a high level of
10 transcription of the gene and expression into gene product. For example, bacterial, phage, or plasmid promoters known in the art from which a high level of transcription has been observed in a host cell system comprising *E. coli* include the lac promoter, trp
15 promoter, recA promoter, ribosomal RNA promoter, the P_R and P_L promoters, lacUV5, ompF, bla, lpp, and the like, may be used to provide transcription of the inserted DNA sequence encoding E amino acid sequences.

20 Additionally, if E protein, E peptides, or E oligopeptides may be lethal or detrimental to the host cells, the host cell strain/line and expression vectors may be chosen such that the action of the promoter is inhibited until specifically induced. For example, in certain operons the addition of specific inducers is
25 necessary for efficient transcription of the inserted DNA (e.g., the lac operon is induced by the addition of lactose or isopropylthio-beta-D-galactoside). A variety of operons such as the trp operon, are under different control mechanisms. The trp operon is induced when
30 tryptophan is absent in the growth media. The P_L promoter can be induced by an increase in temperature of host cells containing a temperature sensitive lambda repressor. In this way, greater than 95% of the promoter-directed transcription may be inhibited in
35 uninduced cells. Thus expression of recombinant E, E peptides, or E oligopeptides may be controlled by

culturing transformed or transfected cells under conditions such that the promoter controlling the expression from the inserted DNA encoding E amino acids is not induced, and when the cells reach a suitable 5 density in the growth medium, the promoter can be induced for expression from the inserted DNA.

Other control elements for efficient gene transcription or message translation include enhancers, and regulatory signals. Enhancer sequences are DNA 10 elements that appear to increase transcriptional efficiency in a manner relatively independent of their position and orientation with respect to a nearby gene. Thus, depending on the host cell expression vector system used, an enhancer may be placed either upstream 15 or downstream from the inserted DNA sequences encoding E amino acids to increase transcriptional efficiency. As illustrated previously in this embodiment, other specific regulatory sequences have been identified which may effect the expression from the gene encoding E. 20 These or other regulatory sites, such as transcription or translation initiation signals, can be used to regulate the expression of the gene encoding E, or gene fragments thereof. Such regulatory elements may be inserted into DNA sequences encoding E amino acids or 25 nearby vector DNA sequences using recombinant DNA methods described herein for insertion of DNA sequences.

Accordingly, *M. catarrhalis* nucleotide sequences containing regions encoding for E, E peptides, or E oligopeptides can be ligated into an expression vector 30 at a specific site in relation to the vector's promoter, control, and regulatory elements so that when the recombinant vector is introduced into the host cell, the *M. catarrhalis* E-specific DNA sequences can be expressed in the host cell. For example, the E-specific DNA 35 sequences containing its own regulatory elements can be ligated into an expression vector in a relation or

orientation to the vector promoter, and control elements which will allow for expression of E amino acid sequences. The recombinant vector is then introduced into the appropriate host cells, and the host cells are 5 selected, and screened for those cells containing the recombinant vector. Selection and screening may be accomplished by methods known in the art including detecting the expression of a marker gene (e.g., drug resistance marker) present in the plasmid, 10 immunoscreening for production of E-specific epitopes using antisera generated to E-specific epitopes, and probing the DNA of the host's cells for E-specific nucleotide sequences using one or more oligonucleotides and methods described according to Embodiment C herein.

15 Genetic engineering techniques may also be used to characterize, modify and/or adapt the encoded E peptides or E proteins. For example, site-directed mutagenesis to modify an outer membrane protein fragment in regions outside the protective domains, may be desirable to 20 increase the solubility of the subfragment to allow for easier purification. Further, genetic engineering techniques can be used to generate DNA sequences encoding a portion of the amino acid sequence of E. For example, from the sequence disclosed as SEQ ID NO:11, it 25 can be determined which restriction enzyme or combination of restriction enzymes may be used to generate sequences encoding E peptides or E oligopeptides. Restriction enzyme selection may be done so as not to destroy the immunopotency of the resultant 30 peptide or oligopeptide. Antigenic sites of a protein may vary in size but can consist of from about 7 to about 14 amino acids. Thus, a protein the size of E may contain many discrete antigenic sites; therefore, many partial gene sequences could encode antigenic epitopes 35 of E. Consequently, using Fig. 1 and SEQ ID NO:11 as guides, restriction enzyme combinations may be used to

generate DNA sequences, which when inserted into the appropriate vector, are capable of directing the production of E-specific amino acid sequences (peptides or oligopeptides) comprising different antigenic epitopes.

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Embodiment B

Conservation of the gene encoding E amongst *M. catarrhalis* strains.

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Previous studies, using antibody adsorption experiments, demonstrated that one or more of the surface-exposed determinants of *M. catarrhalis* were antigenically conserved among most strains (Murphy et al., 1989, *supra*). However, these studies did not address the conservation of the gene encoding E amongst strains. For the nucleotide sequences of the present invention to be useful in diagnostic assays, the gene encoding E must be highly conserved amongst strains of *M. catarrhalis*. In addition, a highly conserved gene indicates that the protein sequence is also highly conserved. For a bacterial protein or peptide to be useful as an antigen in vaccine formulations against infection caused by *M. catarrhalis*, the protein or peptide must contain epitopes that are both immunogenic, and conserved amongst strains of *M. catarrhalis*. To determine the degree of conservation of the gene encoding E among strains of *M. catarrhalis*, genomic DNA was purified and analyzed from 19 isolates recovered from diverse clinical and geographic sources. First, the E-specific gene sequences from the purified DNA of the isolates were amplified using the polymerase chain reaction and primers (SEQ ID NO:16 and SEQ ID NO:17). Analysis of the amplified products, by agarose gel electrophoresis, showed that the gene encoding E was the same size (approximately 1.4 kb) in all the strains

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tested. Additionally, restriction fragment length polymorphisms were analyzed by restricting the amplified products into fragments using either *Hind* III, *Sau*96 I, *Bsl* I, or *Bsg* I, and visualizing the fragments by electrophoresis on a 6% acrylamide gel stained with ethidium bromide. The banding pattern of the amplified products showed no variation among the strains tested with regard to the presence of the restriction sites and minimal differences in the observed size of the fragments (as illustrated in Fig. 2A for *Sau*96 I, and Fig. 2b for *Bsl* I). Of the four different enzymes used in the restriction of the amplified products, three of the enzymes cut at three different sites within the amplified products, and one cuts at two different sites.

Thus, the similar results in all strains indicate that the sequences recognized at the eleven sites are identical among strains tested. The strains listed in Table 1 are the strains tested for restriction fragment length polymorphisms, in the same order as they appear on the gels (shown in Fig. 2A and Fig. 2B, beginning with lane 2). Differences in restriction patterns among different strains may exist, but differences were not seen with these particular restriction enzymes tested.

- 21 -

Table 1.
Isolates of *Moraxella catarrhalis*

	Strain Designation	Clinical Sources
5	Tal 2	sinus
	58	sputum
	3584	middle ear fluid
	9483	middle ear fluid
	45	sputum
	690	sputum
10	621	sputum
	56	sputum
	1	transtracheal aspirate
	42	sputum
	931	sputum
	701	sputum
15	14	sputum
	135	middle ear fluid
	7221	middle ear fluid
	585	blood
	555	middle ear fluid
	25240	ATCC isolate
20	5191	middle ear fluid

25 These findings indicate that the gene encoding E is highly conserved amongst strains of *M. catarrhalis*, and therefore the nucleotide sequences described herein have applications for diagnostic and vaccine use.

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Embodiment C

Methods for using E-specific nucleotide sequences in molecular diagnostic assays for the detection of *M. catarrhalis*.

Because of the conservation of the gene encoding E, as disclosed in Embodiment B, the nucleic acid sequences of the present invention can be used in molecular diagnostic assays for detecting *M. catarrhalis* genetic material. In particular, and as illustrated by SEQ ID NO:1- SEQ ID NO:10 and SEQ ID NO:12- SEQ ID NO:18, E sequence-specific oligonucleotides can be synthesized for use as primers and/or probes in amplifying, and detecting amplified, nucleic acids from *M. catarrhalis*.
Recent advances in molecular biology have provided several means for enzymatically amplifying nucleic acid sequences. Currently the most commonly used method, PCR™ (polymerase chain reaction, Cetus Corporation) involves the use of a thermostable DNA Polymerase, known sequences as primers, and heating cycles which separate the replicating deoxyribonucleic acid (DNA) strands and exponentially amplify a gene of interest. Other amplification methods currently under development include LCR™ (ligase chain reaction, BioTechnica International) which utilizes DNA ligase, and a probe consisting of two halves of a DNA segment that is complementary to the sequence of the DNA to be amplified; enzyme QB replicase (Gene-Trak Systems) and a ribonucleic acid (RNA) sequence template attached to a probe complementary to the DNA to be copied which is used to make a DNA template for exponential production of complementary RNA; and NASBA™ (nucleic acid sequence-based amplification, Cangene Corporation) which can be performed on RNA or DNA as the nucleic acid sequence to be amplified.

Nucleic acid probes that are capable of hybridization with specific gene sequences have been used successfully to detect specific pathogens in biological specimens at levels of sensitivity approaching 10^3 - 10^4 organisms per specimen (1990, Gene Probes for Bacteria, eds. Macario and deMacario,

Academic Press). Coupled with a method that allows for amplification of specific target DNA sequences, species-specific nucleic acid probes can greatly increase the level of sensitivity in detecting organisms in a 5 clinical specimen. Use of these probes may allow direct detection without relying on prior culture and/or conventional biochemical identification techniques. This embodiment of the present invention is directed to primers which amplify species-specific sequences of the 10 gene encoding E of *M. catarrhalis*, and to probes which specifically hybridize with these amplified DNA fragments. By using the nucleic acid sequences of the present invention and according to the methods of the present invention, as few as one *M. catarrhalis* organism 15 may be detected in the presence of 10 µg/ml extraneous DNA.

This embodiment is directed to species-specific oligonucleotides which can be used to amplify sequences of *M. catarrhalis* DNA, if present, from DNA extracted 20 from clinical specimens including middle ear fluid, sputum, blood, and fluids from the nasopharynx, eye, and adenoid; and to subsequently determine if amplification has occurred. In one embodiment of the present invention, a pair of *M. catarrhalis*-specific DNA 25 oligonucleotide primers are used to hybridize to *M. catarrhalis* genomic DNA that may be present in DNA extracted from a clinical specimen, and to amplify the specific segment of genomic DNA between the two flanking primers using enzymatic synthesis and temperature 30 cycling. Each pair of primers are designed to hybridize only to the *M. catarrhalis* nucleotide sequences comprising the gene encoding E (i.e. within the region of the genome containing SEQ ID NO:11) to which they have been synthesized to complement; one to each strand 35 of the double-stranded DNA. Thus, the reaction is specific even in the presence of microgram quantities of

heterologous DNA. For the purposes of this description, the primer derived from the sequence of the positive (gene) strand of DNA will be referred to as the "positive primer", and the primer derived from the 5 sequence of the negative (complementary) strand will be referred to as the "negative primer".

Amplification of DNA may be accomplished by any one of the methods commercially available. For example, the polymerase chain reaction may be used to amplify the 10 DNA. Once the primers have hybridized to opposite strands of the target DNA, the temperature is raised to permit replication of the specific segment of DNA across the region between the two primers by a thermostable DNA polymerase. Then the reaction is thermocycled so that at 15 each cycle the amount of DNA representing the sequences between the two primers is doubled, and specific amplification of the *M. catarrhalis* DNA sequences, if present, results. Further identification of the amplified DNA fragment, as being derived from 20 *M. catarrhalis* DNA, may be accomplished by liquid hybridization. This test utilizes one or more labeled oligonucleotides as probes to specifically hybridize to the amplified segment of *M. catarrhalis* DNA. Detection 25 of the presence of sequence-specific amplified DNA may be accomplished using any one of several methods known in the art such as a gel retardation assay with autoradiography. Thus, the nucleotide sequences of the present invention provide basis for the synthesis of oligonucleotides which have commercial applications in 30 diagnostic kits for the detection of *M. catarrhalis*. In a related embodiment, the oligonucleotides used as primers may be labeled directly, or synthesized to incorporate label. Depending on the label used, the 35 amplification products can then be detected, after binding onto an affinity matrix, using isotopic or colorimetric detection.

DNA may be extracted from clinical specimens which may contain *M. catarrhalis* using methods known in the art. For example, cells contained in the specimen may be washed in TE buffer and pelleted by centrifugation. The 5 cells then may be resuspended in 100 μ l of amplification reaction buffer containing detergents and proteinase K. Using the polymerase chain reaction, the resultant sample may be composed of the cells in 10mM Tris pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01% gelatin, 0.45% NP40TM, 0.045% 10 Tween 20TM, and 60 μ g/ml proteinase K. The sample is incubated at 55°C water bath for 1 hour. Following the incubation, the sample is incubated at 95°C for 10 minutes to heat-inactivate the proteinase K. The sample may then be amplified in accordance with the protocol 15 for the polymerase chain reaction as set forth below.

The *M. catarrhalis* DNA may be amplified using any one of several protocols for amplifying nucleic acids by the polymerase chain reaction. In one mode of this embodiment, the gene encoding E was amplified from 19 20 clinical isolates of *B. catarrhalis* using the following conditions. DNA to be amplified (\sim 1 μ g of genomic DNA) was distributed in 0.5 ml microfuge tubes and the volume was adjusted to 50 μ l by adding a reaction mixture comprising 0.2 mM dNTPs (dATP, dCTP, dGTP, dTTP), 0.25 μ g 25 of each positive and negative oligonucleotide primer, 1 unit of thermostable DNA polymerase, polymerase 10x buffer (5 μ l), 3mM MgSO₄ (final concentration), and sterile distilled water to achieve the total volume. The DNA polymerase is added to the reaction mixture just 30 before use and is gently mixed, not vortexed. A layer of mineral oil, approximately 2 drops, is added to each tube and then the tubes are placed in the thermal cycler. Thirty to thirty-five cycles are generally sufficient for bacterial DNA amplification. One cycle 35 consists of 15 seconds at 96°C, 1 minute at 62°C, and 1

minute at 74°C. The first cycle includes a 3 minute incubation at 95°C to assure complete denaturation.

Oligonucleotides useful as primers or probes which specifically hybridize to the gene encoding E of 5 *M. catarrhalis* and used in DNA amplification and/or detection can be biochemically synthesized, using methods known in the art, from the nucleotide sequences disclosed in the present invention. The specificity of the oligonucleotides for *M. catarrhalis* can be checked 10 by a genebank database (Genbank) search for each individual sequence. In general, the oligonucleotides should be selected for low G-C content. Pairs of primers that have been used for this embodiment to amplify the whole gene encoding E include SEQ ID NO:14 and SEQ ID 15 NO:15. Pairs of primers used to amplify portions of the gene include SEQ ID NO:16 and SEQ ID NO:17; and SEQ ID NO:17 and SEQ ID NO:18.

For detection purposes, the oligonucleotides of the present invention may be end-labeled with a 20 radioisotope. Probe sequences, internal to the two primers used for amplification of the gene sequence, may be end-labeled using T₄ polynucleotide kinase and gamma ³²P ATP. Twenty pMols of probe DNA in kinase buffer (50mM Tris, pH 7.6, 10mM MgCl₂, 5mM dithiothreitol, 0.1mM 25 spermidine-HCl, 0.1mM EDTA, pH 8.0) is mixed with 120 μ Ci of gamma ³²P ATP and incubated at 37°C for 1 hour. Labeled probe is separated from unincorporated label on an 8% acrylamide gel run for 1 hour at 200 volts in Tris Borate EDTA (TBE) buffer at room temperature. Labeled 30 probe is first located by exposing the acrylamide gel to x-ray film for three minutes. The resulting autoradiogram is then positioned under the gel, and the band containing the labeled probe was excised from the gel. The gel slice is pulverized in one milliliter of 35 sterile distilled water, and the probe is eluted by shaker incubation overnight at 37°C. The eluted probe is

separated from the gel fragments by centrifugation using a chromatography prep column. Radioactivity of the probe is determined, by counting one microliter of the labeled probe on a glass fiber filter, by liquid scintillation.

5 Such probe sequences may be chosen from any of the sequences identified as SEQ ID NO: 1 to SEQ ID NO:10, and SEQ ID NO:12 to SEQ ID NO:18 provided the probe sequence is internal to the two primers used for amplification of the desired nucleotide sequence
10 disclosed in the present invention.

Alternative methods known in the art may be used to improve the detection of amplified target sequences in accordance with the compositions and methods of the present invention. The sensitivity of detection of the 15 amplified DNA sequences can be improved by subjecting the sequences to liquid hybridization. Alternative methods of detection known in the art, in addition to gel electrophoresis and gel electrophoresis with Southern hybridization and autoradiography, that may be used with the compositions and methods of the present 20 invention include: restriction enzyme digestion with gel electrophoresis; slot-blot hybridization with a labeled oligonucleotide probe; amplification with a radiolabeled primer with gel electrophoresis, Southern hybridization 25 and autoradiography; amplification with a radiolabeled primer with dot blot and autoradiography; amplification with oligonucleotides containing affinity tags (ex. biotin, or one primer incorporating biotin and the other primer with a sequence specific for a DNA binding 30 protein) followed by detection in an affinity-based assay (ex. ELISA); and amplification with oligonucleotides containing fluorophores followed by fluorescence detection.

One embodiment of non-isotopic detection involves 35 incorporating biotin into the oligonucleotide primers of the present invention. The 5'-aminogroup of the primers

may be biotinylated with sulfo-NHS-biotin, or biotin may be incorporated directly into the primer by synthesizing the primer in the presence of biotin-labeled dNTPs. The non-isotopic labeled primers are then used in amplifying 5 DNA from a clinical specimen. The detection for the presence or absence of amplified target sequences may be accomplished by capturing the amplified target sequences using an affinity matrix having avidin bound thereto, followed by incubation with an avidin conjugate 10 containing an enzyme which can be used to visualize the complex with subsequent substrate development. Alternately, the amplified target sequences may be immobilized by hybridization to the corresponding probes of the target sequence wherein the probes have been 15 affixed onto a matrix. Detection may be accomplished using an avidin conjugate containing an enzyme which can be used to visualize the complex with subsequent substrate development.

20

Embodiment D

Methods for making and using E, E peptides, or E oligopeptides in diagnostic immunoassays.

E protein, E peptides, and E oligopeptides can be 25 purified for use as an immunogen in vaccine formulations; and as an antigen for diagnostic assays or for generating *M. catarrhalis*- specific antisera of therapeutic and/or diagnostic value. E protein from *M. catarrhalis* or peptides thereof, or recombinant E 30 protein, recombinant E peptides, or recombinant E oligopeptides produced from an expression vector system, can be purified with methods known in the art including detergent extraction, chromatography (e.g., ion exchange, affinity, immunoaffinity, or sizing columns), 35 differential centrifugation, differential solubility, or other standard techniques for the purification of

proteins. For example, a partially purified preparation, containing primarily bacterial outer membrane proteins, can be prepared as follows. Bacteria expressing E from 30 chocolate agar plates were scraped into 25 ml of PBS, pH 7.2, and harvested by centrifugation at 12,000 x g for 20 minutes at 4°C. The bacterial pellet was resuspended in 10 ml of 1 M sodium acetate-0.001 M β -mercaptoethanol (pH 4.0). A 90-ml volume of a solution containing 5% Zwittergent Z 3-14 (Calbiochem-Behring) and 0.5% M CaCl₂, was added, and the suspension was mixed for 1 hour at room temperature. Nucleic acids were precipitated by the addition of 25 ml cold ethanol and subsequent centrifugation at 17,000 x g for 10 minutes at 4°C. The remaining proteins were precipitated by the 15 addition of 375 ml cold ethanol and collected by centrifugation at 17,000 x g for 20 minutes at 4°C. The pellets were allowed to dry and were then suspended in 10 ml of detergent buffer containing 0.05% Zwittergent, 0.05 M Tris, 0.01 M EDTA, pH 8.0, and mixed for 1 hour 20 at room temperature. The bacterial outer membrane proteins are present in the soluble fraction of the detergent buffer after centrifugation at 12,000 x g for 10 minutes at 4°C.

Immunopurification of the E protein from an outer 25 membrane protein preparation may be accomplished using methods known in the art for immunoaffinity chromatography. E-specific monoclonal antibodies may be linked to a chromatographic matrix to form an affinity matrix. The outer membrane protein preparation is then 30 incubated with the affinity matrix allowing the antibodies to bind to E. The affinity matrix is then washed to remove unbound components and E is then eluted from the affinity matrix resulting in a purified preparation of E protein. The purified E may be used as 35 an antigen for diagnostic assays, or may be chemically or enzymatically cleaved into peptides using methods

known to those in the art. Alternatively, E peptides, or oligopeptides, may be chemically synthesized using the deduced amino acid sequence from the gene encoding E as a reference. Recombinant E protein may be purified using 5 similar methods.

E oligopeptides are defined herein as a series of peptides corresponding to a portion of the amino acid sequence of E protein as disclosed in SEQ ID NO:11 that are synthesized as one or chemically-linked. Such 10 peptides or oligopeptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid peptide synthesis using tert-butyloxycarbonyl amino acids (Mitchell et al., 1978, *J. Org. Chem.* 43:2845-2852), using 9- 15 fluorenylmethyloxycarbonyl amino acids on a polyamide support (Dryland et al., 1986, *J. Chem. So. Perkin Trans. I*, 125-137); by pepscan synthesis (Geysen et al., 1987, *J. Immunol. Methods* 03:259; 1984, *Proc. Natl. Acad. Sci. USA* 81:3998); or by standard liquid phase 20 peptide synthesis. Modification of the peptides or oligopeptides, such as by deletion and substitution of amino acids (and including extensions and additions to amino acids) and in other ways, may be made so as to not substantially detract from the immunological properties 25 of the peptide or oligopeptide. In particular, the amino acid sequence of the E protein may be altered by replacing one or more amino acids with functionally equivalent amino acids resulting in an alteration which is silent in terms of an observed difference in the 30 physicochemical behavior of the protein, peptide, or oligopeptide.

Purified E protein, E peptides, and E oligopeptides may be used as antigens in immunoassays for the detection of *Moraxella catarrhalis*-specific antisera 35 present in the body fluid of an individual suspected of having an infection caused by *M. catarrhalis*. The body

fluids include, but are not limited to, middle ear fluid, sputum, blood, and fluids from the nasopharynx, eye, and adenoid. The detection of E, E peptides, or E oligopeptides as an antigen in immunoassays, includes
5 any immunoassay known in the art including, but not limited to, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), "sandwich" assay, precipitin reaction, agglutination assay, fluorescent immunoassay, and chemiluminescence-based immunoassay.
10

Embodiment E

Methods and compounds for vaccine formulations related to E, E peptides, and E oligopeptides.

15 This embodiment of the present invention is to provide E protein and/or peptides thereof, to be used in as immunogens in a prophylactic and/or therapeutic vaccine for active immunization to protect against or treat infections caused by *M. catarrhalis*. For vaccine development, the E-specific amino acid sequences comprising the immunogen may be purified from *M. catarrhalis* or may be purified from a host containing a recombinant vector which expresses E, E peptides, or E oligopeptides. Such hosts include, but are not limited
20 to, bacterial transformants, yeast transformants, filamentous fungal transformants, and cultured cells that have been either infected or transfected with a vector which encodes E amino acid sequences. Peptides or oligopeptides corresponding to portions of the E protein
25 may be produced from chemical or enzymatic cleavage of E protein, or chemically synthesized using methods known in the art and with the amino acid sequence deduced from the nucleotide sequence of the gene encoding E as a reference. Alternatively, E peptides or E oligopeptides
30 may be produced from a recombinant vector. In either case, the E protein, E peptide or E oligopeptide
35

immunogen is included as the relevant immunogenic material in the vaccine formulation, and in therapeutically effective amounts, to induce an immune response. Many methods are known for the introduction of
5 a vaccine formulation into the human or animal to be vaccinated. These include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration. The vaccine may further comprise a
10 physiological carrier such as a solution, a polymer or liposomes; and an adjuvant, or a combination thereof.

Various adjuvants are used in conjunction with vaccine formulations. The adjuvants aid in attaining a more durable and higher level of immunity using smaller
15 amounts of vaccine antigen or fewer doses than if the vaccine antigen were administered alone. The mechanism of adjuvant action is complex and not completely understood. However, it may involve immunomodulation through the stimulation of cytokine production,
20 phagocytosis and other activities of the reticuloendothelial system, as well as delayed release and degradation/processing of the antigen to enhance immune recognition. Examples of adjuvants include incomplete Freund's adjuvant, Adjuvant 65 (containing
25 peanut oil, mannide monooleate and aluminum monostearate), oil emulsions, Ribi adjuvant, the pluronic polyols, polyamines, Avridine, Quil A, saponin, MPL, QS-21, and mineral gels such as aluminum hydroxide, aluminium phosphate, etc.

30 Another embodiment of this mode of the invention involves the production of E-specific amino acid sequences as a hapten, i.e. a molecule which cannot by itself elicit an immune response. In such case, the hapten may be covalently bound to a carrier or other
35 immunogenic molecule which will confer immunogenicity to the coupled hapten when exposed to the immune system.

Thus, such a E-specific hapten liked to a carrier molecule may be the immunogen in a vaccine formulation.

Another mode of this embodiment provides for either a live recombinant viral vaccine, recombinant bacterial vaccine, recombinant attenuated bacterial vaccine, or an inactivated recombinant viral vaccine which is used to protect against infections caused by *M. catarrhalis*.

Vaccinia virus is the best known example, in the art, of an infectious virus that is engineered to express

vaccine antigens derived from other organisms. The recombinant live *vaccinia virus*, which is attenuated or otherwise treated so that it does not cause disease by itself, is used to immunize the host. Subsequent replication of the recombinant virus within the host

provides a continual stimulation of the immune system with the vaccine antigens such as E, or E peptides, thereby providing long-lasting immunity. Other live vaccine vectors include: adenovirus, cytomegalovirus, and preferably the poxviruses such as *vaccinia* (Paoletti

and Panicali, U.S. Patent No. 4,603,112) and attenuated *Salmonella* strains (Stocker et al., U.S. Patent Nos. 5,210,035; 4,837,151; and 4,735,801; and Curtiss et al., 1988, *Vaccine* 6:155-160). Live vaccines are

particularly advantageous because they continually

stimulate the immune system which can confer substantially long-lasting immunity. When the immune response is protective against subsequent *M. catarrhalis* infection, the live vaccine itself may be used in a preventative vaccine against *M. catarrhalis*.

To illustrate this mode of the embodiment, using molecular biological techniques such as those illustrated in Embodiment A, the gene encoding E, or a gene fragment encoding one or more E peptides may be inserted into the *vaccinia virus* genomic DNA at a site which allows for expression of E epitopes but does not negatively affect the growth or replication of the

vaccinia virus vector. The resultant recombinant virus can be used as the immunogen in a vaccine formulation. The same methods can be used to construct an inactivated recombinant viral vaccine formulation except that the 5 recombinant virus is inactivated, such as by chemical means known in the art, prior to use as an immunogen and without substantially affecting the immunogenicity of the expressed immunogen. A mixture of inactivated viruses which express different epitopes may be used in 10 the formulation of a multivalent inactivated vaccine. In either case, the inactivated recombinant vaccine or mixture of inactivated viruses may be formulated with a suitable adjuvant in order to enhance the immunological response to the vaccine antigens.

15 In another variation of this embodiment, genetic material is used directly as the vaccine formulation. Nucleic acid (DNA or RNA) containing sequences encoding E, E peptide or E oligopeptide, operatively linked to one or more regulatory elements can be introduced 20 directly to vaccinate the individual ("direct gene transfer") against pathogenic strains of *M. catarrhalis*. Direct gene transfer into a vaccinated individual, resulting in expression of the genetic material by the vaccinated individual's cells such as vascular 25 endothelial cells as well as the tissue of the major organs, has been demonstrated by techniques in the art such as by injecting intravenously an expression plasmid:cationic liposome complex (Zhu et al., 1993, *Science* 261:209-211). Other effective methods for 30 delivering vector DNA into a target cell are known in the art. In one example, purified recombinant plasmid DNA containing viral genes has been used to inoculate (whether parentally, mucosally, or via gene-gun immunization) vaccines to induce a protective immune 35 response (Fynan et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11478-11482). In another example, cells removed from

an individual can be transfected or electroporated by standard procedures known in the art, resulting in the introduction of the recombinant vector DNA into the target cell. Cells containing the recombinant vector DNA 5 may then be selected for using methods known in the art such as via a selection marker expressed in the vector, and the selected cells may then be re-introduced into the individual to express E protein, E peptide, or E oligopeptide.

One preferred method of vaccination with genetic material comprises the step of administering to the individual the nucleic acid molecule that comprises a nucleic acid sequence that encodes for one or more of the E protein, E peptides, or E oligopeptides, wherein 10 the nucleic acid molecule is operatively linked to one or more regulatory sequences necessary for expression. The nucleic acid molecule can be administered directly, or first introduced into a viral vector and administered via the vector. The nucleic acid molecule can be 15 administered in a pharmaceutically acceptable carrier or diluent and may contain compounds that can enhance the effectiveness of the vaccine. These additional compounds include, but are not limited to, adjuvants that modulate and enhance the immune response, or other compounds 20 which increase the uptake of nucleic acid by the cells. The immunization with the nucleic acid molecule can be 25 through any parental route (intravenous, intraperitoneal, intradermal, subcutaneous, or intramuscular), or via contact with mucosal surfaces of 30 the nasopharynx, trachea, or gastrointestinal tract.

As an alternative to active immunization, such as where an immunocompromised individual is suffering from a potentially life-threatening infection caused by *M. catarrhalis*, immunization may be passive, i.e. 35 immunization comprising administration of purified human immunoglobulin containing antibody against E epitopes.

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It should be understood that while the invention has been described in detail herein, the examples were for illustrative purposes only. Other modifications of the embodiments of the present invention that are 5 obvious to those skilled in the art of molecular biology, medical diagnostics, and related disciplines are intended to be within the scope of the appended claims.

- 37 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Murphy, Timothy F.
Bhushan, Reva

5 (ii) TITLE OF INVENTION: Vaccine for *Moraxella catarrhalis*

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:
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15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Kb storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: MS-DOS/ Microsoft Windows 3.1
(D) SOFTWARE: Wordperfect

20 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: U.S. Serial No. 08/245,758
(B) FILING DATE: 17/05/94

25 (vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Nelson, M. Bud
(B) REGISTRATION NUMBER: 35,300
(C) REFERENCE DOCKET NUMBER: 11520.0063

(viii) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1 :

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium

45 (v) SEQUENCE DESCRIPTION: SEQ ID NO:1 :

CAAGATGGTA CATATGCGAA 20

(3) INFORMATION FOR SEQ ID NO:2 :

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
5 (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:2 :

10 CAAGATGGTA CGTATGCGAA 20

(4) INFORMATION FOR SEQ ID NO:3 :
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
20 (iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
25 (v) SEQUENCE DESCRIPTION: SEQ ID NO:3 :

CAAGATGGTA CTTATGCGAA 20

(5) INFORMATION FOR SEQ ID NO:4 :
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
35 (ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
40 (C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:4 :

CAAGATGGTA CCTATGCGAA 20

(6) INFORMATION FOR SEQ ID NO:5 :
45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
50 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*

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(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:5 :

5 CAAGATGGCA CATATGCGAA 20

(7) INFORMATION FOR SEQ ID NO:6 :
(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
15 (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:6 :

20 CAAGATGGCA CGTATGCGAA 20

(8) INFORMATION FOR SEQ ID NO:7 :
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
30 (iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
35 (v) SEQUENCE DESCRIPTION: SEQ ID NO:7 :

CAAGATGGCA CTTATGCGAA 20

(9) INFORMATION FOR SEQ ID NO:8 :
40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
45 (ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
50 (C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:8 :

CAAGATGGCA CCTATGCGAA 20

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(10) INFORMATION FOR SEQ ID NO:9 :

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 nucleotides
(B) TYPE: nucleic acid
5 (C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
10 (A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:9 :

15 GGCTTGGGCA ACTTTGTCAT CACCCTCC 28

(11) INFORMATION FOR SEQ ID NO:10 :

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 nucleotides
20 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
25 (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:10 :

30 GTTGAATTCA CACCAAGTTG AAAATCCAAG 30

(12) INFORMATION FOR SEQ ID NO:11 :

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1650 nucleotides
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: double-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
40 (iii) HYPOTHETICAL: yes
(iv) IMMEDIATE SOURCE:
(A) LIBRARY: genomic
(B) CLONE: EMBL-3 clone
(v) ORIGINAL SOURCE:
45 (A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(vi) FEATURE:
(A) LOCATION: E gene region, 154-1531
50 (vii) SEQUENCE DESCRIPTION: SEQ ID NO:11 :

TAAACGCATA AAAATTGTAA GAAAATATAT ATATTTACT TGTTTGTA 50

TTAAATTCA TTTAAGATAC AAATGTGTAA GACTTTGTA CTGTTCTATA 100

	AAGAAGTATG GACAGTTTA CATATTGTAA GGACTGACTT TTTGGAGAAA	150
	GTG ATG AGC TTA AAA TTT GGA TAC AAA GCG CTG AGT TTG GCG	192
5	Met Ser Leu Lys Phe Gly Tyr Lys Ala Leu Ser Leu Ala	
	1 5 10	
	GTA TTT TCA ACC CTA ACC GCA ACC GCA GCA CAA GCA GCA GGC	234
	Val Phe Ser Thr Leu Thr Ala Thr Ala Ala Gln Ala Ala Gly	
10	15 20 25	
	CTG GAT CGC TCA GGG CAA GAT GTG ACT GCT TTT TTA CAA GAT	276
	Leu Asp Arg Ser Gly Gln Asp Val Thr Ala Phe Leu Gln Asp	
	30 35 40	
15	GGC ACT TAT GCC GAA ACC GTT TAT ACT TAT ATT GAT GCC AAT	318
	Gly Thr Tyr Ala Glu Thr Val Tyr Thr Tyr Ile Asp Ala Asn	
	45 50 55	
20	GTT ACC GGT AAA GAT ACC GCA GGC AAA GAT ACA GGT GAT ATT	360
	Val Thr Gly Lys Asp Thr Ala Gly Lys Asp Thr Gly Asp Ile	
	60 65	
25	GCC GAA GCT TAT GAT TTT TTC CGT TAC GGT GTT AAA GCA GAC	402
	Ala Glu Ala Tyr Asp Phe Phe Arg Tyr Gly Val Lys Ala Asp	
	70 75 80	
30	ATC AAC GAC ACC TTT AGC ATC GGT GTG CTA TAT GAC GAG CCA	444
	Ile Asn Asp Thr Phe Ser Ile Gly Val Leu Tyr Asp Glu Pro	
	85 90 95	
	TTT GGT GCA GCG GTT CAA TAT GAC GGT AAT AGT AAT TTT GTG	486
	Phe Gly Ala Ala Val Gln Tyr Asp Gly Asn Ser Asn Phe Val	
	100 105 110	
35	GCA GAT AAA AAT GCA ACA GCA ACA ATT TTT GCC CAA GCT ATC	528
	Ala Asp Lys Asn Ala Thr Ala Thr Ile Phe Ala Gln Ala Ile	
	115 120 125	
40	AAT CAG GCT ACA AAA GCA CAA TTA AAC GAT AGC CTT GCT TAT	570
	Asn Gln Ala Thr Lys Ala Gln Leu Asn Asp Ser Leu Ala Tyr	
	130 135	
45	AAA TCA ATT AAG CCA GTT TTA GAC AGT GTT AAA TCA CCT CAG	612
	Lys Ser Ile Lys Pro Val Leu Asp Ser Val Lys Ser Pro Gln	
	140 145 150	
50	CGT GCT TTG GCA GTA GCA TCA ATC GTA GAA ACC AAT TCA GCA	654
	Arg Ala Leu Ala Val Ala Ser Ile Val Glu Thr Asn Ser Ala	
	155 160 165	
	CAA GCC AAA CCC ATT GCT GAC CGA TTA AGA GCA GCG GCT GCA	696
	Gln Ala Lys Pro Ile Ala Asp Arg Leu Arg Ala Ala Ala Ala	
	170 175 180	

	CAT GCA GAA GCA ACT GAC GGT CAA AAG ACT AAT GTC GAA ATT	738
	His Ala Glu Ala Thr Asp Gly Gln Lys Thr Asn Val Glu Ile	
	185 190 195	
5	CGC ACC AAC AAC CTA ACC ATG TTA GTC GGT GCC AAA TTG GGT	780
	Arg Thr Asn Asn Leu Thr Met Leu Val Gly Ala Lys Leu Gly	
	200 205	
10	GCT AAT AAA AAT TTC CAA ATC TAT GGC GGT CCT GTG GCT CAA	822
	Ala Asn Lys Asn Phe Gln Ile Tyr Gly Gly Pro Val Ala Gln	
	210 215 220	
15	AGA GTT AAG GGC GAA GTG CAT TTG CGT GGT CCT GCT TAT CAA	864
	Arg Val Lys Gly Glu Val His Leu Arg Gly Pro Ala Tyr Gln	
	225 230 235	
20	GTC ATG ACA GGT TAT GAT GCC AAA ATT GCA ACA GAT ACT CAA	906
	Val Met Thr Gly Tyr Asp Ala Lys Ile Ala Thr Asp Thr Gln	
	240 245 250	
25	TTG GGC TGG GCG GCA GGT TTG GCA TTT TAT AAA CCC GAA ATT	948
	Leu Gly Trp Ala Ala Gly Leu Ala Phe Tyr Lys Pro Glu Ile	
	255 260 265	
30	GCC CTA AAA GCC GCT TTG ACC TAT CGC TCT GAG ATT GAG CAT	990
	Ala Leu Lys Ala Ala Leu Thr Tyr Arg Ser Glu Ile Glu His	
	270 275	
35	GAC TCT GAA ATT GCC GAA ACC ATT CCT GTT ACG GGC TAT GCG	1032
	Asp Ser Glu Ile Ala Glu Thr Ile Pro Val Thr Gly Tyr Ala	
	280 285 290	
40	GGT AAA AAG GAT TTT AAA GTT ACT TTG CCT GAC TCA TGG AAC	1074
	Gly Lys Lys Asp Phe Lys Val Thr Leu Pro Asp Ser Trp Asn	
	295 300 305	
45	TTA GAT TTT CAA ACT GGT GTG AAT CCA ACA ACG CTA TTA ACT	1116
	Leu Asp Phe Gln Thr Gly Val Asn Pro Thr Thr Leu Leu Thr	
	310 315 320	
50	GCC AAA GTA CGC TAT GTA CCA TGG TCT GAT TTT GAC ATT CGC	1158
	Ala Lys Val Arg Tyr Val Pro Trp Ser Asp Phe Asp Ile Arg	
	325 330 335	
45	CCA ACA CAG TAT ACA GAA ACC ACA AAA CTT CGT TAT CCA CAG	1200
	Pro Thr Gln Tyr Thr Glu Thr Thr Lys Leu Arg Tyr Pro Gln	
	340 345	
50	GGT TTA CCA ATC ATC AGC TAT GAC AAA GAC CAA TGG TCG GCT	1242
	Gly Leu Pro Ile Ile Ser Tyr Asp Lys Asp Gln Trp Ser Ala	
	355 360	

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	GAA	GTT	GGT	TTG	GGT	AAG	CGT	GTT	AGC	GAT	CGT	TTG	GCT	GTT	1284		
	Glu	Val	Gly	Leu	Gly	Lys	Arg	Val	Ser	Asp	Arg	Leu	Ala	Val			
	365							370				375					
5	TCA	GGT	GCG	GTA	GGT	TGG	GAT	AGT	GGT	GCA	GGT	AAC	CCT	GCA	1326		
	Ser	Gly	Ala	Val	Gly	Trp	Asp	Ser	Gly	Ala	Gly	Asn	Pro	Ala			
	380			385					385			390					
10	AGT	AGC	TTA	GGT	CCT	ATC	AAA	GGC	TAT	TAT	TCA	TTG	GGC	TTA	1368		
	Ser	Ser	Leu	Gly	Pro	Ile	Lys	Gly	Tyr	Tyr	Ser	Leu	Gly	Leu			
	395				400				395			405					
15	GGT	GCG	CGG	TAT	AAT	GTT	ACA	CCT	GAA	TGG	TCG	CTG	TCT	TTG	1410		
	Gly	Ala	Arg	Tyr	Asn	Val	Thr	Pro	Glu	Trp	Ser	Leu	Ser	Leu			
	410				415				410			420					
20	GGT	GGT	AAA	TAC	TTT	AAA	TTT	GGA	GAT	GCT	CAA	GCA	CAG	CTA	1452		
	Gly	Gly	Lys	Tyr	Phe	Lys	Phe	Gly	Asp	Ala	Gln	Ala	Gln	Leu			
	425					430				425							
	CCA	ACC	AAA	GAT	AAA	GTA	GGT	AAC	TTT	GAT	AGT	AAT	GAT	GGC	1494		
	Pro	Thr	Lys	Asp	Lys	Val	Gly	Asn	Phe	Asp	Ser	Asn	Asp	Gly			
	435				440				435			445					
25	TAT	GCC	TTG	GGC	GTT	AAG	CTT	GCT	TAT	CAC	GCC	AAA	TAATCT	1536			
	Tyr	Ala	Leu	Gly	Val	Lys	Leu	Ala	Tyr	His	Ala	Lys					
	450					455				450		460					
30	CATGCTAAAT	CATA	AAAAAA	TGTCTAAATA	TAAAAAA	ATAG	CTTGAATTTC								1586		
	AAGCTATT	TTATTAGTTG	GTTAAA	ATT	AACGAAT	CTC	AACCGTCGCA								1636		
	CATTTCGATG	ACAG															
35	(13) INFORMATION FOR SEQ ID NO:12 :																
	(i)	SEQUENCE CHARACTERISTICS:															
		(A)	LENGTH: 24 nucleotides														
		(B)	TYPE: nucleic acid														
		(C)	STRANDEDNESS: single-stranded														
40			(D)	TOPOLOGY: linear													
	(ii)	MOLECULE TYPE: DNA															
	(iii)	IMMEDIATE SOURCE: synthesized															
	(iv)	ORIGINAL SOURCE:															
		(A)	ORGANISM: <i>Moraxella catarrhalis</i>														
45		(B)	STRAIN: 25240														
		(C)	CELL TYPE: bacterium														
	(v)	SEQUENCE DESCRIPTION: SEQ ID NO:12 :															
50	CGCCAAACTC AGCGCTTGT ATCC 24																
	(14) INFORMATION FOR SEQ ID NO:13 :																
	(i)	SEQUENCE CHARACTERISTICS:															
		(A)	LENGTH: 24 nucleotides														
		(B)	TYPE: nucleic acid														

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(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(iii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
5 (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:13 :

10 GTCAGTCCTT CCAATATGTA AAAC 24

(15) INFORMATION FOR SEQ ID NO:14 :
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
20 (iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
25 (v) SEQUENCE DESCRIPTION: SEQ ID NO:14 :

CGCATAAAAAA TTGTAAGAAA ATATATATAT TTTAC 35

(16) INFORMATION FOR SEQ ID NO:15 :
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
35 (ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
40 (C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:15 :

GCTATTTTTT ATATTTAGAC ATTTTGAT GATTTAGC 38

45 (17) INFORMATION FOR SEQ ID NO:16 :
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
50 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*

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(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:16 :

5 GTGATGAGCT TAAAATTGG ATACAAAGCG CTGAG 35

(18) INFORMATION FOR SEQ ID NO:17 :
(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 30 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) IMMEDIATE SOURCE: synthesized
15 (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
(v) SEQUENCE DESCRIPTION: SEQ ID NO:17 :

20 GCATGAGATT ATTTGGCGTG ATAAGCAAGC 30

(19) INFORMATION FOR SEQ ID NO:18 :
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 34 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
30 (iii) IMMEDIATE SOURCE: synthesized
(iv) ORIGINAL SOURCE:
(A) ORGANISM: *Moraxella catarrhalis*
(B) STRAIN: 25240
(C) CELL TYPE: bacterium
35 (v) SEQUENCE DESCRIPTION: SEQ ID NO:18 :

GCAGGCCTGG ATCGCTCAGG GCAAGATGTG ACTG 34

What is claimed is:

1. A vaccine formulation comprising an immunologically effective amount of a substantially pure peptide,
5 oligopeptide, or protein having one or more epitopes of E, wherein E is an outer membrane protein of *Moraxella catarrhalis* of an apparent molecular mass of from about 35,000 to about 50,000 daltons by SDS-PAGE and having an amino acid sequence substantially as
10 depicted in SEQ ID NO:11 from amino acid residue 26 to 460.
2. The vaccine formulation according to claim 1, in which the peptide, oligopeptide, or protein was produced
15 recombinantly from cells cultured from a host cell system genetically engineered to include a vector containing a nucleotide sequence that regulates expression of DNA sequences encoding E epitopes, said host cell system is selected from the group consisting
20 of bacteria, yeast, filamentous fungi, insect cell lines, and mammalian cell lines.
3. The vaccine formulation according to claim 1, in which the peptide or oligopeptide is produced by
25 chemical or enzymatic cleavage of E protein.
4. The vaccine formulation according to claim 1, in which the peptide or oligopeptide is produced by chemical synthesis.
30
5. The vaccine formulation of claim 1, further comprising a pharmaceutical carrier.
6. The vaccine formulation according to claim 2, in
35 which the cultured cell is a bacterium.

7. The vaccine formulation according to claim 2, in which the cultured cell is a yeast.
8. The vaccine formulation according to claim 2, in
5 which the cultured cell is a filamentous fungus.
9. The vaccine formulation according to claim 2, in which the cultured cell is an insect cell line.
10. 10. The vaccine formulation according to claim 2, in which the cultured cell is a mammalian cell line.
11. A substantially pure antigenic peptide, oligopeptide, or protein having one or more epitopes of
15 E, wherein E is an outer membrane protein of *Moraxella catarrhalis* of an apparent molecular mass of from about 35,000 to about 50,000 daltons by SDS-PAGE and having an amino acid sequence substantially as depicted in SEQ ID NO:11 from amino acid residue 26 to
20 460.
12. The peptide, oligopeptide or protein according to claim 11, in which the peptide, oligopeptide, or protein was produced recombinantly from cells cultured from a
25 host cell system genetically engineered to include a vector containing a nucleotide sequence that regulates expression of DNA sequences encoding E epitopes, said host cell system is selected from the group consisting of bacteria, yeast, filamentous fungi, insect cell
30 lines, and mammalian cell lines.
13. The peptide, oligopeptide or protein according to claim 12, in which the cultured cell is a bacterium.
- 35 14. The peptide, oligopeptide or protein according to claim 12, in which the cultured cell is a yeast.

15. The peptide, oligopeptide or protein according to
claim 12, in which the cultured cell is a filamentous
fungus.

5 16. The peptide, oligopeptide or protein according to
claim 12, in which the cultured cell is an insect cell
line.

10 17. The peptide, oligopeptide or protein according to
claim 12, in which the cultured cell is a mammalian cell
line.

15 18. A recombinant vector comprising a DNA sequence
encoding one or more antigenic determinants or epitopes
of E, wherein E is an outer membrane protein of
15 *Moraxella catarrhalis* of an apparent molecular mass of
from about 35,000 to about 50,000 daltons by SDS-PAGE
and having an amino acid sequence substantially as
depicted in SEQ ID NO:11 from amino acid residue 26 to
20 460.

19. The recombinant vector of claim 18, wherein the
vector is selected from the group consisting of a
plasmid vector, phagemid vector, cosmid vector, and a
25 viral vector.

20. A composition useful to passively immunize
individuals suffering from an infection caused by *M.*
catarrhalis, said composition comprising purified
30 antiserum which recognizes one or more epitopes of E,
wherein E is an outer membrane protein of *M. catarrhalis*
of an apparent molecular mass of from about 35,000 to
about 50,000 daltons by SDS-PAGE and having an amino
acid sequence substantially as depicted in SEQ ID NO:11
35 from amino acid residue 26 to 460.

21. Purified oligonucleotides useful in the detection of *M. catarrhalis*, said oligonucleotides consisting essentially of nucleic acid sequences which complement and specifically hybridize to conserved regions of the 5 gene comprising the 1377 base pair open reading frame of SEQ ID NO:11 or its corresponding complementary strand.

22. The oligonucleotides of claim 21, wherein the oligonucleotides consist essentially of nucleic acid 10 sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ 15 ID NO:18.

23. A method for detecting the presence or absence of *Moraxella catarrhalis* in a clinical specimen, wherein the method comprises the steps of:

20

- (a) lysing *Moraxella catarrhalis* cells in the specimen to release bacterial genetic material;
- (b) contacting the genetic material with two 25 oligonucleotides under suitable conditions permitting hybridization of the oligonucleotides to the genetic material, wherein a first oligonucleotide hybridizes to a region within a gene comprising the 1377 base pair open reading frame of SEQ ID NO:11, and a second oligonucleotide hybridizes to a region in the corresponding complementary strand;
- (c) enzymatically amplifying a specific region of 30 sequence of the genetic material comprising the gene and its corresponding strand using

35

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the oligonucleotides of step (b) as primers;
and

(d) detecting the presence of amplified sequences
of the gene and its corresponding strand,
5 wherein the presence of these amplified
sequences correlate to the presence of *M.*
catarrhalis in the specimen.

24. The method of claim 23, wherein the detection is
10 further facilitated by hybridization of amplified
sequences with a labeled oligonucleotide probe
consisting of a nucleotide sequence corresponding to a
region in the amplified sequence, if present, said label
is a label known to be incorporated in oligonucleotides
15 selected particularly from among radioactive label, such
as ^{32}P , and enzymatic label, such as biotin.

25. The method of claim 23, wherein the specimen is a
body fluid selected from the group consisting of middle
20 ear fluid; sputum; blood; and fluids from the
nasopharynx, or eye, or adenoid.

26. A method for the detection of *Moraxella catarrhalis*
in a clinical specimen, wherein the method comprises the
25 steps of:

(a) obtaining a specimen of body fluid;
(b) lysing the *Moraxella catarrhalis* cells in the
specimen to release bacterial genetic
30 material;
(c) contacting the genetic material with an
oligonucleotide probe synthesized to
correspond to a region of a gene comprising
the 1377 base pair open reading frame of SEQ
35 ID NO:11, or its corresponding complementary
strand under suitable conditions permitting

hybridization of the oligonucleotide to the genetic material; and

5 (d) detecting interaction between the specimen and the probe, said interaction being between the genetic material of *M. catarrhalis* and the probe.

27. The method of claim 26, wherein the specimen is a body fluid selected from the group consisting of middle 10 ear fluid; sputum; blood; and fluids from the nasopharynx, or eye, or adenoid.

28. A method for the detection of *M. catarrhalis*-specific antisera in a body fluid of an individual 15 comprising using peptide or protein having one or more epitopes of E as an antigen in an immunoassay to interact with and detect *M. catarrhalis*-specific antisera in the body fluid, wherein E is an outer membrane protein of *Moraxella catarrhalis* of an apparent 20 molecular mass of from about 35,000 to about 50,000 daltons by SDS-PAGE and having an amino acid sequence substantially as depicted in SEQ ID NO:11 from amino acid residue 26 to 460.

25 29. The method of claim 28, wherein the immunoassay is an assay selected from the group consisting of a radioimmunoassay, enzyme-linked immunosorbent assay, "sandwich" assay, precipitin reaction, agglutination assay, fluorescent-based immunoassay, and 30 chemiluminescence-based immunoassay.

30. An isolated gene or fragments thereof encoding epitopes of outer membrane protein E of *Moraxella catarrhalis* having an apparent molecular mass 35 of from about 35,000 to about 50,000 daltons by SDS-

PAGE, wherein said gene comprises the 1377 base pair open reading frame of SEQ ID NO:11.

31. A vaccine formulation comprising a nucleic acid
5 molecule which encodes the E protein, or one or more
gene fragments that encodes one or more E peptides or E
oligopeptides, said nucleic acid molecule is operatively
linked with regulatory sequences; and a pharmaceutically
acceptable carrier or diluent.

10

32. An infectious, recombinant microorganism capable of
expressing E protein, E peptides or E oligopeptides, of
M. catarrhalis.

15 33. A microorganism of claim 32, which is a vaccinia
virus, adenovirus, or cytomegalovirus.

34. A microorganism of claim 32, which is a bacterium
of the genus *Salmonella*.

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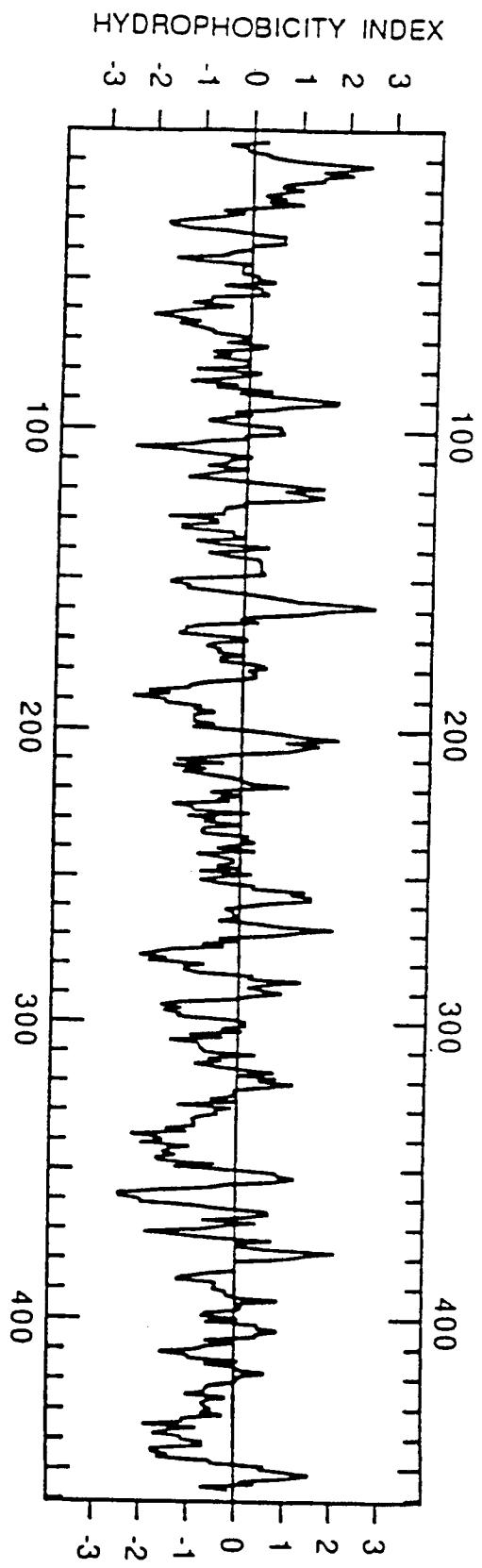


FIG. 1

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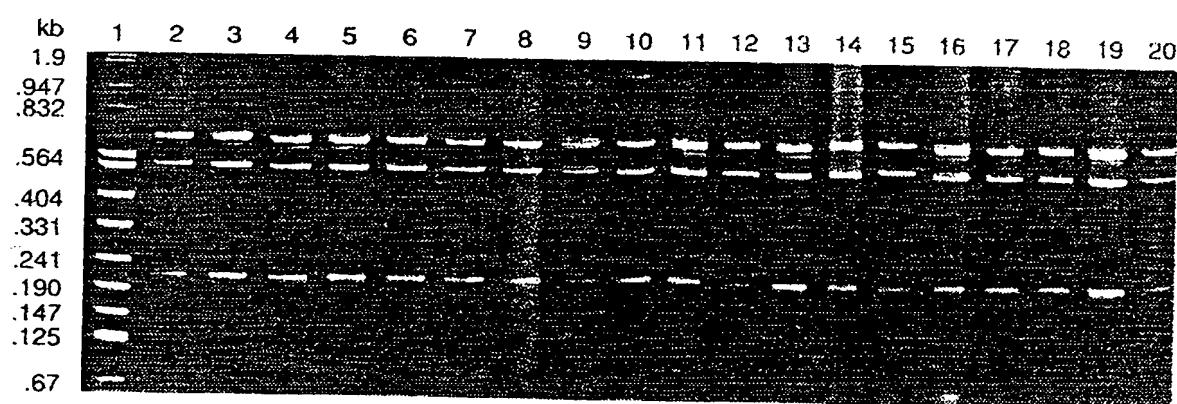


FIG. 2A

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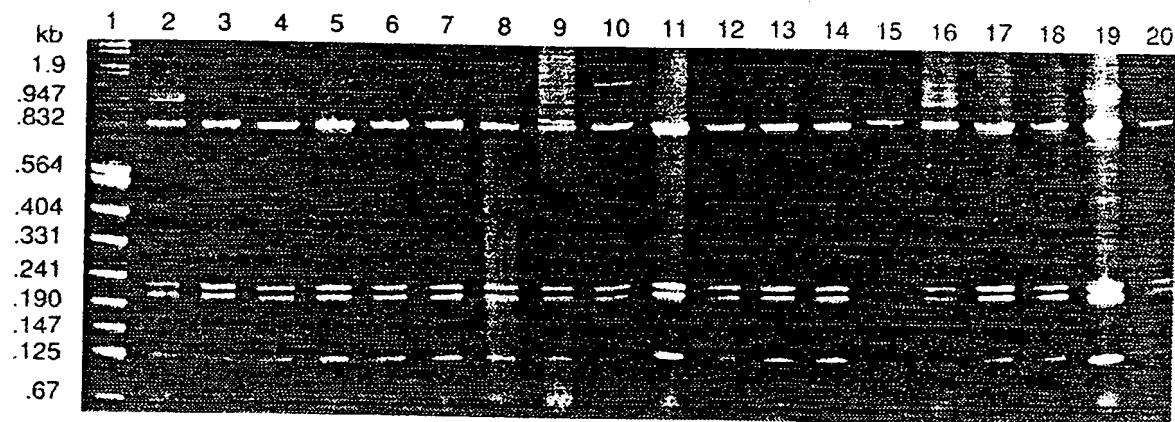


FIG. 2B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/05134

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1; 435/6, 7.1 and 320.1; 530/380; 536/23.1 and 24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular Microbiology, Volume 10, No. 1, issued 1993, T.F. Murphy et al, "The Major Heat-Modifiable Outer Membrane Protein CD is Highly Conserved Among Strains of Branhamella catarrhalis, pages 87-97, see entire document.	1-18,21,22 and 30-34
X	Infection and Immunity, Volume 60, No. 3, issued March 1992, J. Sarwar et al, "Characterization of an Antigenically Conserved Heat-Modifiable Major Outer Membrane Protein of Branhamella catarrhalis, pages 804-809, see entire document.	1-17,20,28, 29 and 31

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/05134

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Infection and Immunity, Volume 62, No.4, issued April 1994, K.L. Klingman et al, "Purification and Characterization of a High-Molecular-Weight Outer Membrane Protein of Moraxella (Branhamella) catarrhalis, pages 1150-1155, see entire document.	1-17,20,28,29 and 31
X	US, A, 4,659,678 (FORREST ET AL) 21 April 1987, col. 7, line 40 to col. 10, line 44.	28 and 29
X	US, A, 5,030,556 (BEAULIEU ET AL) 09 July 1991, col. 8, line 5 to col. 9, line 19.	26 and 27
X	US, A, 4,983,511, (GEIGER ET AL) 08 January 1991, col. col. 7, line 20 to col. 8, line 41.	23-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/05134

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/00, 39/38, 35/14, 38/16, 31/70; C12Q 1/68; G01N 33/53; C12N 15/70; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/184.1; 435/6, 7.1 and 320.1; 530/380; 536/23.1 and 24.5; 514/44